FORMULATION AND EVALUATION OF TOPICAL DRUG DELIVERY SYSTEM CONTAINING CLOBETASOL PROPIONATE NIOSOMES

Dissertation submitted to

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CERTIFICATE

This is to certify that the Dissertation entitled **"FORMULATION AND EVALUATION OF TOPICAL DRUG DELIVERY SYSTEM CONTAINING CLOBETASOL PROPIONATE NIOSOMES"** submitted by M. Abraham Lingan in partial fulfillment of the requirement for the award of degree of **Master of Pharmacy in Pharmaceutics** is a bonafide work carried out by him under my guidance and supervision during the academic year 2007-2008 in the Department of Pharmaceutics, Madurai Medical College, Madurai-625020, affiliated to **The Tamilnadu Dr.M.G.R.Medical University, Chennai.**

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(M.SEKAR BABU)

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INTRODUCTION

Records of medicines and treatment in this world stretch back to more than 4500 years. For most of the past centuries the physicians have little real knowledge, relied mainly on superstition and herbal cures. Knowledge about drugs and diseases starts promptly before 300 years and currently there is a drastic change in their development.[1]

Even today conventional drug delivery system occupies most of the part in a prescription as well as drug store. Even though they release the drug promptly, there are some disadvantages.[2]

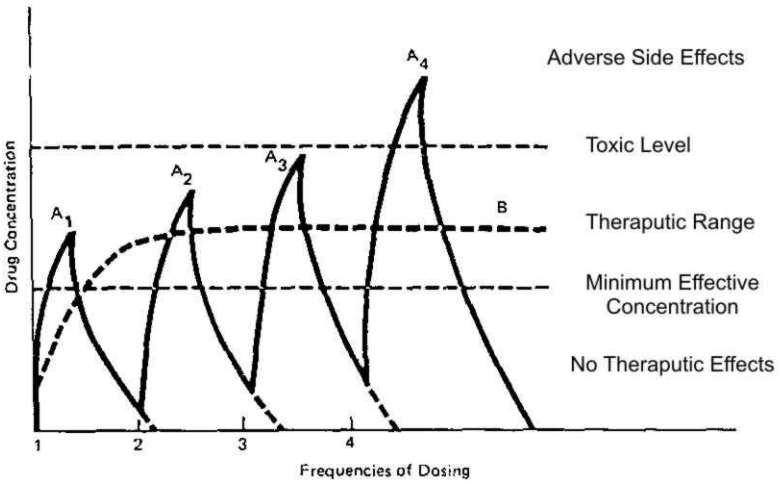
DISADVANTAGES OF CONVENTIONAL DRUG DELIVERY[2]

1) Slow action.
2) Frequent uptake of drug may lead to patient non compliance.
3) Targeting or discontinuance of dose may lead to less (or) prolonged drug therapy.
4) Costly.

To over come the above disadvantages new technologies of drug delivery have emerged in the field.

They are as follows[2],

1. Sustained Release.
2. Controlled Release.
3. Targeted Delivery.



Hypothetical drug concentration profiles in the systemic circulation resulting from the consecutive administration of multiple doses of an immediate-release drug delivery system (A_1, A_2, . . .) compared to the ideal drug concentration profile (B) required for treatment.

Figure - 1

1) Sustained Release

Sustained release means the appearance of the drug in systemic circulation is delayed and/or prolonged and its plasma profile is sustained in duration. i.e.; onset of pharmacological action is delayed but therapeutic effect is sustained.

2) Controlled Release

Controlled drug delivery release is the rate of drug delivery which is predictable and also the release rate is reproducible from one unit to another.

3) Targeted delivery

The targeted drug delivery means the delivery of drugs by systemic administration as drug – carrier complex in the aim of drug delivery at a particular diseased cell (or) tissue (or) organ[2].

CONTROLLED DRUG DELIVERY SYSTEM

To achieve and maintain the concentration of administered drug within therapeutically effective range, it is often necessary to take drug dosage several times and these results are in a fluctuating drug levels in plasma. Controlled drug delivery systems are introduced in order to over come the above disadvantage of the conventional drug delivery system[3].

Controlled drug delivery systems are the dosage forms which are developed to achieve better patient compliance, modified drug release, delivery of drug at the site of action, more efficient administration of drugs by various routes and for better therapeutic effect[4].

CLASSIFICATION OF CONTROLLED DRUG DELIVERY SYSTEMS[5,6]

1. Rate pre-programmed drug delivery system.
2. Activation modulated drug delivery system.
3. Feed back regulated drug delivery system.
4. Site targeting drug delivery system.

1. Rate Pre Programmed Drug Delivery System

Here in this system the release of drug molecules from the delivery system has been preprogrammed at specific rate profiles.

Types:

a. Polymer Membrane Permeation – Controlled Drug Delivery System.
b. Polymer Matrix Diffusion – Controlled Drug Delivery System.
c. Microreservoir Partition – Controlled Drug Delivery System.

2. Activation Modulated Drug Delivery System

In this group of controlled drug delivery system the release of drug from the delivery device is activated by physical, chemical (or) bio-chemical process and/or facilitated by the energy supplied externally.

Types

A) Physical means

a. Osmotic pressure – Activated Drug Delivery System.
b. Hydrodynamic pressure – Activated Drug Delivery System.
c. Vapour pressure – Activated Drug Delivery System.
d. Mechanically – Activated Drug Delivery System.
e. Magnetically – Activated Drug Delivery System.
f. Sonophoresis – Activated Drug Delivery System.

g. Ionophoresis – Activated Drug Delivery System.

h. Hydration – Activated Drug Delivery System.

B. chemical means

i. pH – Activated Drug Delivery System.

ii. Ion - Activated Drug Delivery System.

iii. Hydrolysis – Activated Drug Delivery System.

C. biochemical means

i. Enzyme – Activated Drug Delivery System.

ii. Biochemical – Activated Drug Delivery System.

3. Feed back – regulated drug delivery system

In this group of system the release of drug molecules from the delivery system is activated by a triggering agent, such as a biochemical substance in the body and also regulated by its concentration via some feed back mechanisms.

a. Bioerosion – regulated Drug Delivery System.

b. Bioresponsive Drug Delivery System.

c. Self regulated Drug Delivery System.

4. Site Targeting Drug Delivery System[7]

Here in this system the drugs are particularly delivered at the targeted cell, tissue or the organ. The techniques of drug targeting are utilization of carriers such as;

a. Liposomes,
b. Niosomes,
c. Microspheres,
d. Nanoparticles,
e. Antibodies,
f. Cellular carriers and
g. Macromolecules.

ADVANTAGES OF CONTROLLED DRUG DELIVERY SYSTEM[6]

1. Employ less total drug, optimize therapy and improved patient compliance,
2. Minimize (or) eliminate local side effects and drug accumulation with chronic dosing,
3. Obtain less potential or reduction in drug activity with chronic use,
4. Improve control of condition i.e; reduce fluctuation in drug level, improve bioavailability and treatment efficiencies of some drugs,

5. Make use of specific drugs e.g; sustained release aspirin for morning relief of arthritis by dosing earlier,
6. Maintenance of optimum therapeutic drug concentration in the blood with minimum fluctuations,
7. Predictable and reproducible release rates for extended duration,
8. Enhancement of activity duration for short half life drug,
9. Elimination of frequent dosing, wastage of drug and inconvenience of night time administration of drug.
10. Reduction of the incidence, degree of toxicity, side effects and irritation of GI tract caused by some orally administered drugs.

DISADVANTAGES OF CONTROLLED DRUG DELIVERY SYSTEM[8]

1. High cost.
2. Unpredictable (or) poor in-vitro – in-vivo correlation.
3. Dose dumping.
4. Reduce potential for dosage adjustment.
5. Increase first pass clearance.
6. Poor systemic availability in general.

FACTORS INFLUENCING THE DESIGN AND PERFORMANCE OF CONTROLLED DRUG DELIVERY SYSTEMS[6,8]

1) Drug Properties / Physiochemical Properties

- Partition co-efficient.
- Drug Stability.
- Protein binding.
- Molecular size and diffusivity.
- Aqueous solubility.

2) Biological Properties

- Absorption.
- Distribution.
- Metabolism.
- Elimination and biological half-life.
- Dose size.
- Route of drug delivery.
- Target sites.
- Acute or chronic therapy.
- The pathological disease.
- The patient condition.
- Duration of action.

- Margin of safety.
- Circadian rhythm.

3) Physiological Properties

- Prolonged drug absorption.
- Variability in GI Emptying and motility.
- Gastro Intestinal Blood flow.

4) Pharmacokinetic Properties

- Dose dumping.
- First Pass metabolism.
- Variability of urinary pH effect on drug elimination.
- Enzyme induction/inhibition upon multiple dosing.

5) Pharmacological properties

- Changes in drug effect upon multiple dosing.
- Sensitizing / tolerance.

DRUGS UNSUITABLE FOR CONTROLLED DRUG DELIVERY[8]

1. Short / long elimination half life.
2. Narrow therapeutic index.
3. Poor absorption.
4. Active absorption.

5. Large doses.
6. Low aqueous s solubility.
7. Extensive first pass metabolism.

FUTURE TRENDS IN CONTROLLED DRUG DELIVERY SYSTEM[8]

The most exciting and challenging opportunities in controlled drug delivery lie in the arena of responsive delivery system, with which it will be possible to deliver drug through implantable devices in response to a measured blood level and to deliver the drug precisely to a target size.

NIOSOMES – A REVIEW

CHAPTER III

NIOSOMES

Paul enlrich, who coined the term 'Magic bullet' in early 20th century, where a carrier system was proposed to simply carry to its site of action and releasing it selectively while non-target sites should absolutely be exempted from drug effect. This concept is proved in case of liposomes[9].

Liposomes were discovered in the early 1960's by Bangham and colleagues and subsequently became the most extensively explored drug delivery system[10]. Liposomes are concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of natural (or) synthetic phospholipids and cholesterol.

But there are some limitations in Liposomal delivery system.

DISADVANTAGES OF LIPOSOMES[10,11]

1. Liposomal phospholipid undergo chemical degradation such as oxidation and hydrolysis.
2. Liposomes in aqueous suspension may aggregate, fuse (or) leak their contents.
3. Cost of natural phospholipids are usually high.
4. Purity of natural phospholipids is always doubtful.
5. Due to oxidative degradation they should be stored in nitrogen atmosphere.
6. The phospholipids will interact with serum components (High density lipoprotein). These high density lipoprotein will remove the phospholipids from the vesicles and lead to leakage of drugs.
7. Liposomal formulations are expensive.

Due to the above demerits of liposomes, a new vesicular system emerged known as Niosomes.

NIOSOMES (Non Ionic Surfactant Vesicle)

Niosomes are non-ionic surfactant vesicles first initiated by Ballie et.al in 1985. Niosomes are concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of non-ionic surfactants and cholesterol.

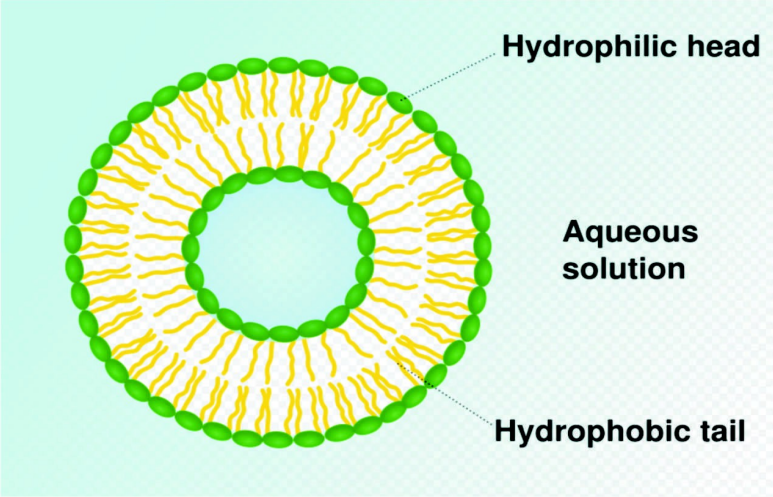


Figure - 2

ADVANTAGES OF NIOSOMES[7,10,11]

1. Stability of niosomes are better than liposomes.
2. Niosomes can prolong the circulation of entrapped drugs.
3. Due to the presence of non-ionic surfactant, the targeting nature of drugs to brain and liver and also for tumour are better.
4. So due to the presence of better targeting nature it is proved that usage of niosomal technology in treating cancer, parasitic, viral and other microbial disease are more effective.
5. Non-ionic surfactant will increase both fluidity and permeation of biological membranes.
6. Niosomes are non-ionic and also biodegradable.
7. Niosomes reduce systemic toxicity of drugs such as anti-cancer, anti-infectives etc.

8. As a carrier for enhanced drug delivery to specific cells they improve the therapeutic index by restricting the drug effects to target cells.
9. Niosomes could serve as intramuscular cargo and depots for short acting peptide drugs which are cleared fast from the circulation.
10. These systems are potent stimulators of the cellular and humoral immue response.
11. The formulation of antigen as a niosome in w/o emulsion further increases the activity of antigens.
12. Compared to liposomes, usage of non-ionic surfactant instead of phospholipids have better chemical stability, precise in chemical composition and cheaper in cost.
13. Niosomes can enhance the skin penetration of drugs.

TYPES OF NIOSOMES[11]

They are divided intro three types. They are as follows;

a) Small Niosomes (100 nm to 200 nm)
b) Large Niosomes (800 nm to 900 nm)
c) Big Niosomes (2µm to 4 µm)

BASIC COMPONENTS OF NIOSOMES

1. Non-ionic surfactant (or) Amphiphilies.
2. Cholesterol.
3. Drug.
4. Distilled water (or) Buffer.

METHODS OF PREPARATION[7,10,12,13]

1. Hand Shaking Method.
2. Ether Injection Method.
3. Sonication Method.
4. Reverse Phase Evaporation Method.
5. Aqueous Dispersion Method.
6. Extrusion Method.
7. Microfludization Method.
8. Thin Film Hydration Method.
9. Transmembrane pH Gradient Method.

1) Hand Shaking Method

In this method surfactant and cholesterol are mixed in organic solvent and the organic layer is transferred to round bottom flask by vacuum. Then it is allowed to evaporate in round bottomed flask and upon hydration the surfactant swells to form vesicles.

2) Ether Injection Method

Here the vesicles are formed, when the surfactant: Cholesterol mixture in organic solvent is slowly injected by a 14 guaze needle at a rate of 0.25 ml/min in aqueous phase maintained at 60^0C.

3) Sonication Method

In this method surfactant: Cholesterol mixture was dispersed in a 2 ml of aqueous phase in a vial. Then the dispersion was probe sonicated for 3 minutes at 60^0C.

4) Reverse phase evaporation method

Surface active agents are dissolved in chloroform and 0.25 volume of phosphate buffer saline is emulsified to get w/o emulsion. The mixture is then sonicated and subsequently chloroform is evaporated under reduced pressure. The lipid (or) surfactant forms a gel first and subsequently hydrates to form vesicles.

5) Aqueous Dispersion Method

This method essentially based on microdispersion of surfactants in aqueous media containing solutes for encapsulation (or) entrapment. Continuous agitation under controlled temperature condition leads to homogenous vesiculation.

6) Extrution Method

Niosomes were prepared by using C16 Cr2 a chemically defined non-ionic surfactant by extrution through a Polycarbonate membrane (0.1 mm nucleopore)

7) Microfludization Method

This method is based on submerged jet principle in which two fluidized streams interact at ultra high velocities (upto 1700 ft/sec) in precisely defined micro channels within the interaction chamber. The impingement of thin liquid sheets along a common front is arranged such that the energy supplied to the system remains with in the area of niosome formulation.

8) Thin Film Hydration Method

The surfactant : Cholesterol mixture is dissolved in volatile organic solvent and the solvent is evaporated in round bottom flask at 60^0C until a thin film was formed. Then aqueous solvent is added and vortexed for 1 hour. The film peels off and later swells to form niosomes.

9) Transmembrane pH gradient Method

Here the surfactant and cholesterol were dissolved in chloroform. The solvent was evaporated on the walls of round bottom flask. The film

is hydrated with 300 mm citric acid by vortex mixing. Then the product was frozen. To this niosomal suspension, aqueous solution was added and vortexed. The pH of the solution was raised to 7.0 to 7.2 with 1ml of disodium hydrogen phosphate. The mixture was later heated at 60^0C for 10 minutes.

SIZE, SHAPE AND MORPHOLOGY[10,13,14]

Vesicular structure of surfactant based vesicles has been visualized and established using freeze fracture electron microscopy while photon correlation spectroscopy could be successfully used to determine mean diameter of the vesicles.

The vesicles can also be examined using light microscope, polarized light microscope, Olympus microscope, infrared spectroscopy and electron microscopy.

ENTRAPMENT EFFICIENCY [10]

For finding entrapment efficiency the vesicles have to be lysed and made upto the volume by suitable solvent. For the vesicular disruption the following chemicals can be used:

- Hydrophilic surfactant solulan C24
- Triton X-100

- 0.2% desoxycholate
- 50% propanol

The Entrapment efficiency can be found out by the following formula.

Entrapment efficiency = % Drug content - % of maximum drug release of unentrapped drug.

Effect Of Cholesterol In Niosomes[16]

Cholesterol plays an important role in absolute encapsulation efficiency of the formulation and in turn the stability of niosomes.

However after certain concentration the intercalation of cholesterol in the bilayers decreases the entrapment volume and thus entrapment efficiency. Therefore increase in the concentration of cholesterol decreases the entrapment efficiency.

And also after certain concentration the increase in cholesterol content decreases the maximum rigidity of niosomal membrane properties.

Effect Of Surfactant In Niosomes[17]

Surfactants are '**Surfac**e Ac**t**ing **A**g**e**n**ts**' (wetting agents) which lowers the surface tension of a liquid.

Various surfactants are used in the niosomal preparation are Span's, Tween's and Brij's.

The mean size of niosomes increases when increase in HLB value of span. [Span 85 to span 20 respectively].

Entrapment efficiency increases when increase in phase transition temperature. Since phase transition temperature of Span 60 was greater than Span 40, Span 60 shows more entrapment efficiency when compared with Span 40.

The entrapment efficiency increases with increase in the concentration and lipophilicity of the surfactant. But the entrapment efficiency of Span 80 was less when compared with Span 60, this is because of the presence of unsaturated alkyl chain in Span 80.

STABILITY OF NIOSOMES[10]

Studies showed that the niosomal preparations are stable comparatively than the liposomal preparations.

Osmotic Effect[15]

Addition of hypertonic salt solution to suspension of niosomes brings about reduction in vesicle diameter with concominant water efflux, which may be due to pumping out of vesicle content where as in hypotonic salt solution there is initial slow release with slight swelling of vesciles probably due to inhibition of eluting fluid from vesicles followed by faster release, which may due to mechanical loosening of vesilces under osmotic stress.

Stability In Vivo

Moser and colleagues 1980 studied plasma protein interaction with haemoglobin containing niosomes. There was no agglutination. Haemoglobin niosomes are found to be stable, however they are chemically instable, i.e; oxidation of haemoglobin to methaemoglobin was recorded as a result of haemoglobin content reached about 30% after 5 months at 4^0C

TOXICITY OF NIOSOMES

There was no toxic reports yet filed due to the usage of niosomes as drug carriers in animals. But however the toxic effects are directly related to the type of drug released.

LIST OF NIOSOMALY ENTRAPPED BIO ACTIVE AGENTS[10]

Various bioactive agents which are entrapped in niosomes are sodium stilbogluconate, Methotrexate, vincristine, doxorubicin, diclofenac sodium, bovine serum albumin, s9-Desglycinamide 8-Arginine vasopressin, insulin, estradiol, antipyrine, rifampicin, Haemoglobin.

APPLICATIONS OF NIOSOMES[10,16,18]

Niosomal drug delivery is potentially applicable to many pharmacological agents for their action against diseases. By niosomal formation we can potentially deliver both hydrophobic (or) amphiphilic drugs.

1. Targeting of bioactive agents to reticulo endothelial system and to organs other than reticulo endothelial system.
2. Used in Neoplasia.
3. Used in Leishmaniasis.
4. Used to delivery peptide drugs.
5. Used in immunological applications.
6. Used as a carrier of haemoglobin.
7. Used in Transdermal drug delivery of drugs.
8. Used in Diagnostic imaging.
9. Used in oral drug delivery.
10. Useful in sustained release and localized drug action.

CHAPTER – IV

TRANSDERMAL DRUG DELIVERY SYSTEM

INTRODUCTION

Transdermal drug delivery system is one among of the various modes of drug delivery which facilitates passage of therapeutic quantities of drug substances through the skin and for systemic (or) local effects[19].

Discovering a new medicine is a very expensive and time-consuming work. But however, redesigning the modules and means to transport medicine into the body is a less demanding and more lucrative task.

In the normal drug release, if the medication may not be absorbed means it will release too slowly (or) if it delivered too fastly means, the patient may suffer untoward effects. To rectify the above drawback one of the solutions developed was transdermal drug delivery systems[20].

SKIN[21]

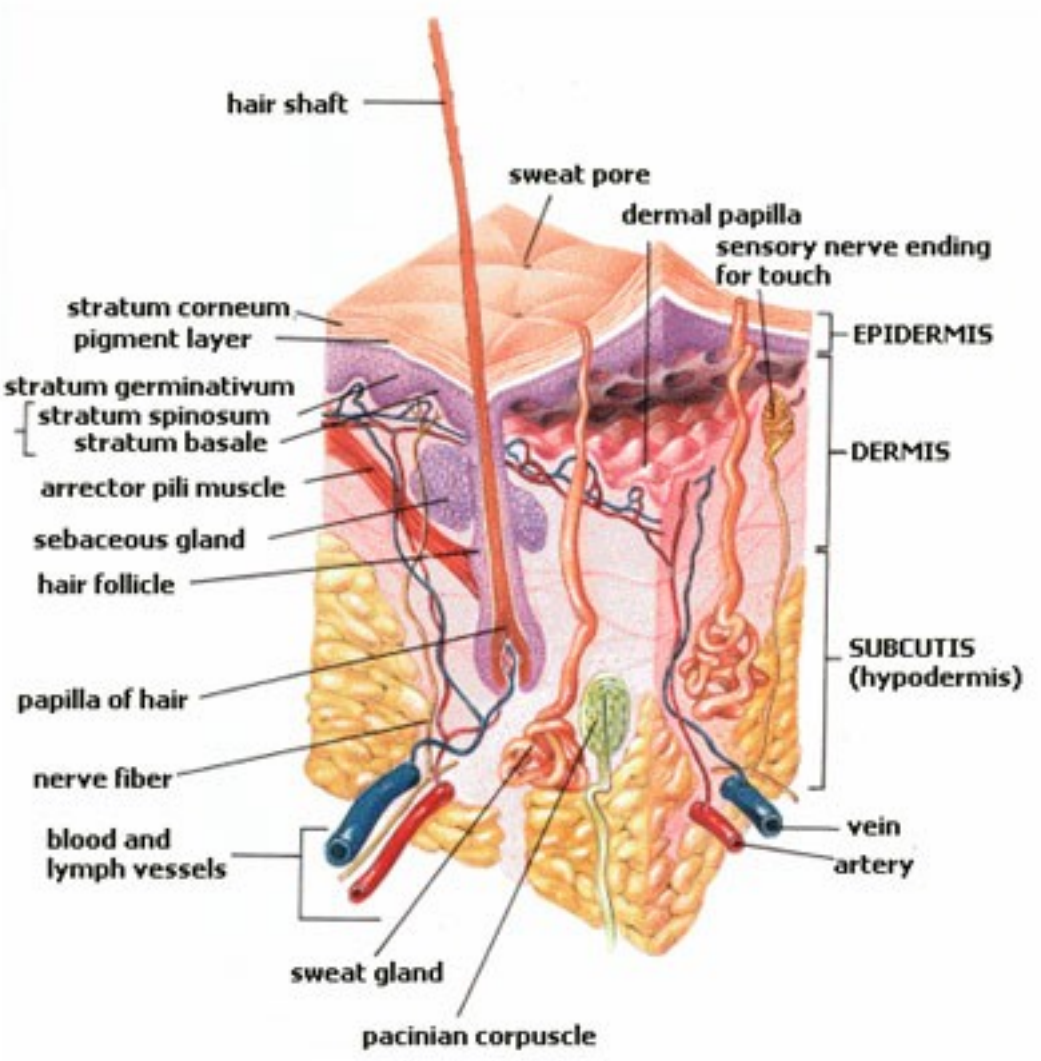


Figure - 3

Skin is the largest organ of 1.5 to 2 m^2 in adult which covers the whole body. Thickness of skin varies from place to place i.e; it is so thick in palm, foot and so thin in eyelid.

The skin is broadly classified into two layers. They are; A) epidermis and B) Dermis.

A. Epidermis

It is the most superficial (or) outermost layer of skin. The cells in the epidermis shed periodically and replaced by new cells usually a complete replacement of epidermis takes about 40 days.

Various Layers In Epidermis

There are about four layers. They are;

i. Stratum corneum.

ii. Stratum lucidum.

iii. Stratum granulosm.

iv. Germinative layer.

B. Dermis

Dermis consists of the following things in it

- Blood vessels.
- Lymph vessels.
- Sensory (somatic) nerve ending.
- Sweat glands and their ducts.
- Hair roots, hair follicles and hairs.
- The arrectores pilorum – involuntary muscles attached to the hair follicles.
- Sebaceous glands.

Heirs, secretions from sebaceous glands and ducts of sweat glands pass via the epidermis to reach the surface.

FUNCTION OF SKIN

It does major functions to the human body. They are;

- Mechanical function
- Protective function
    - microbiological barrier
    - Chemical barrier
    - Radiation barrier
    - Electrical Barrier
- Regulation of body temperature
- Formation of vitamin D
- Sensation
- Absorption
- Excretion

RATIONAL APPROACH TO DELIVER THE DRUG VIA SKIN[22]

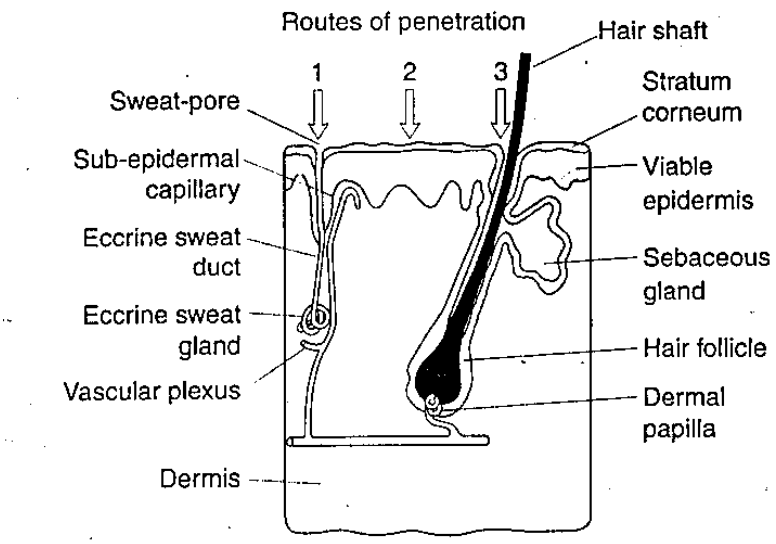
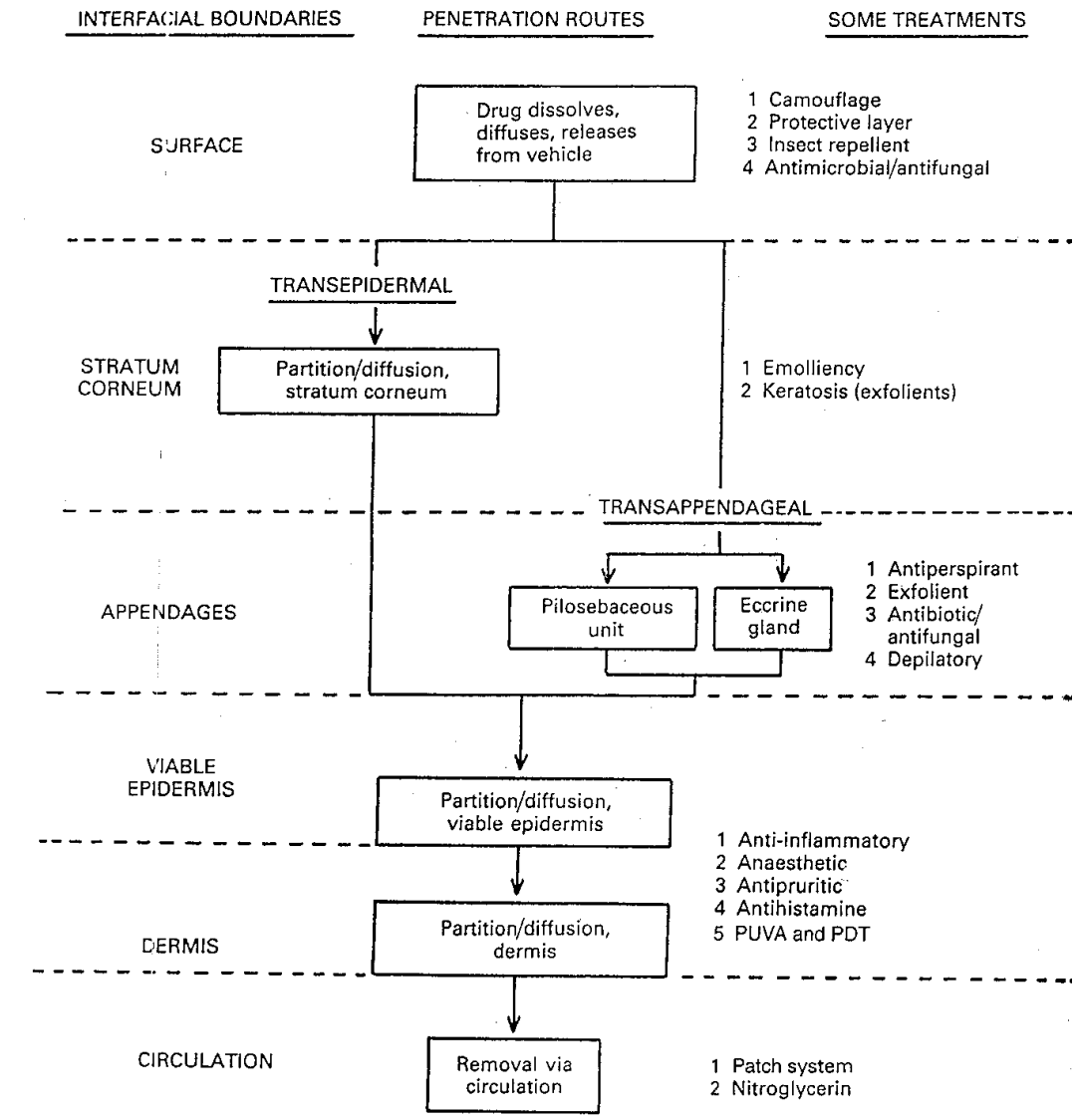


Figure - 4

There are three main ways to approach the problem of formulating a successful topical dosage form.

1. Manipulating the barrier function of the skin
2. Directing drugs to the viable skin tissues without using oral, systemic (or) other routes.
3. Using skin delivery for systemic treatment.

Dermatologist aim five main target regions. They are skin surface, horny layer, viable epidermis & upper dermis, skin glands and systemic circulation.



The macroroutes by which drugs penetrate the skin and examples of treatments appropriate to disorders of the various strata

Figure – 5

FACTORS AFFECTING DRUG DELIVERY

Transdermal route should have the capability to deliver the drug, regardless of size (or) structure at a predetermined rate. But there are some factors which influence the rate of drug delivery. There are two types of factors

They are as follows

A. Biological factors

- Skin condition.
- Skin age.
- Amount of blood flow.
- Regional Skin sites.
- Skin metabolism.
- Species differences.

B. Physiochemical Factors

- Skin hydration.
- Temperature and pH
- Diffusion Coefficient
- Drug applying surface area.
- Drug Concentration.
- Partition Coefficient.
- Molecular size and shape.
-

TYPES OF TREATMENT ACHIEVED BY TOPICAL DRUG DELIVERY

- Camouflage.
- Protection effects,.
- Insect repellant.
- Antimicrobial.
- Antifungal.
- Emolliency.
- Keratosis.
- Antiperspirant.
- Exfolient.
- Antibiotic.
- Depilatory.
- Anti inflammation.
- Anti pruritic.
- Local anesthetic.
- PUFA and PDT.
- Anti histamine.
- Anti angina.
- Anti-ischaemic.

VARIOUS TYPE OF DOSAGE FROM USED IN TOPICAL DRUG DELIVERY

- Liquid preparations.
- Gels (jellies).
- Powders.
- Ointments.
- Creams.
- Paste.
- Aerosols.
- Poultice.
- Transdermal patch.

METHODS TO INCREASE PERCUTANEOUS ABSORPTION[20]

Chemical penetration enhancers such as –

- Solvents
- Alkyl Methyl sulphoxides
- pyrrolidones
- Azone and related compounds
- surfactants

- Ionophoresis
- Sonophoresis
- Electrophoresis

- Laser Ablation

- prodrugs

ADVANTAGES OF TRANSDERMAL DRUG DELIVERY SYSTEM[20]

1. Avoidance of significant presystemic metabolism (degredation in GI tract (or) Liver, gut wall) and the daily drug dose is reduced.
2. Reduction of Inter and Intra Patient variability.
3. Drug level can be maintained in systemic circulation within the therapeutic window.
4. Drug action is extended and frequency of administration is reduced.
5. Improved patient compliance.
6. Drug input can be easily terminated.
7. Increased safety.
8. Greater convenience.
9. Drugs with short biological half lives.

DISADVANTAGES OF TRANSDERMAL DRUG DELIVERY SYSTEM[20]

1. Skin irritation.
2. Skin allergy.
3. Potent drugs cannot be given by this route.

ROLE OF NIOSOMES IN TRANSDERMAL DRUG DELIVERY SYSTEM[23]

Niosomes can be used to deliver both hydrophobic and hydrophilic drugs via transdermal route. Although niosomes were tried for various routes it is used in the market for transdermal route (Novasome Products Such as 30% Petrolatum Novasomes and 10% Salicyclic Acid Novasomes). Studies showed that an enhanced delivery of drugs when encapsulated in niosomes. Niosomes increase skin penetration of drugs and it can act as local depot for sustained release of dermally active compounds. When non ionic surfactants are incorporated into niosomes they are much better tolerated by the skin then when they are used in emulsion.

VARIOUS BIO ACTIVE AGENTS WHICH ARE TRIED VIA TRANS DERMAL ROUTE AS NIOSOME DRUG DELIVERY SYSTEM[23,24,25,26,27,28,29]

- Cyclosporin – A.
- Lidocaine.
- Estradiol.
- Erythromycin.
- Alpha – interferon.
- Diclofenac sodium.

- Nimesulide.
- Enoxacin.
- Miconazole nitrate.
- Ketoconazole.
- Tretionin.
- Metronidazole.

CHAPTER – V

TOPICAL CORTICOSTEROIDS

CORTICOSTEROIDS

INTRODUCTION [21]

The adrenal (or) supra renal glands present above each kidney secrete the steroidal hormones. Two types of steroidal hormones are Glucocorticosteroids and mineralocorticosteroids

HISTORY OF CORTICOSTEROIDS[30,31]

By the middle of 19th century it was demonstrated that adrenal glands were essential for life. A number of steroidal active principles were isolated and their structures were elucidated by Kendall and his coworkers in 1930s. However isolation of "cortisone" by Kendall, Reichstein and Hench was a remarkable event in corticosteroids and they got noble prize for medicine in 1950.

General Chemical Structure of corticosteroids

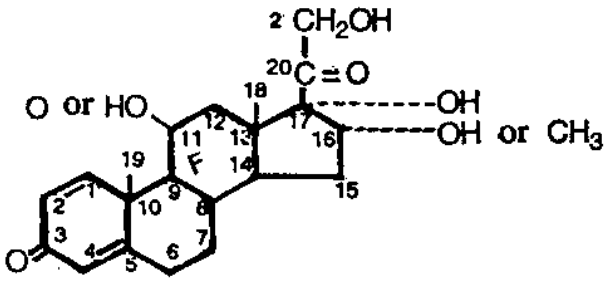


Figure – 6

ACTIONS OF GLUCOCORTICOSTEROIDS[31]

1. Effects on carbohydrate metabolism.
2. Effects on protein metabolism.
3. Effects on fat metabolism.
4. Effects on calcium metabolism.
5. Effects on water excretion.
6. It restricts capillary permeability and maintains tone of arterioles & myocardial contractibility.
7. Effects on skeletal muscles for normal muscular activity.
8. Mild euphoria is quite common with glucocorticosteroids.
9. It increases the gastric acid and pepsin secretion.
10. It increases the number of red blood cells, platelets and neutrophils in circulation.
11. Irrespective of the type and cause of injury the glucocorticoids will suppress the inflammatory response.
12. It will suppress all types of hyper sensitization and allergic phenomena.

TOPICAL CORTICOSTEROIDS[32]

INTRODUCTION

With the introduction of topical steroid for the treatment of skin diseases, the phase of dermatological therapy changed drastically. From

the period of Sulzberger and Witten, experiments had started to increase the potency and to reduce the side effects. This resulted in the introduction of a number of molecules. As the search is going on and the "ideal" topical steroid has not been found, newer and newer molecules are being introduced one after another.

HISTORY

The development from the discovery of the structure of cholesterol, the precursor of all steroids, to the first application of a topically effective steroid in 1952 it took about 20 years. Encouraged by this success of systemically administered corticosteroids, people started to experiment with topical application of steroid. The first molecule they tried was cortisone acetate, which was a miserable failure. During this time Merck & Co. Made "Compound F" and Sulzberger and Witten incorporated it in an ointment base (25 mg of base). The 'Compound F' was hydrocortisone acetate. They used this ointment in a number of conditions including atopic dermatitis, psoriasis, chronic discoid lupus erythematosus, chronic lichenoid dermatoses, pemphigus vulgaris, and alopecia areata. The reports of this trial appeared in the journal of investigative dermatology in 1952 and were the turning point in the history of topical therapy in dermatology. Since then newer and newer molecules were added with

variations in efficacy and side effect profile and now we have super potent corticosteroid.

PHARMACOLOGY

The clinical effects of topical corticosteroids depend on the structure of the molecule, the vehicle and the skin onto which it is applied.

All steroids have the basic 4-ring structure of cholesterol, with three hexane ring and one pentane ring. Modifications in the basic 4-ring structure result in various agents with differing solubility, lipophilicity, percutaneous absorption and glucocortiocoid receptor binding activity. Addition of a fluorine molecule at positions 6 and / or 9 enhances the potency of steroid. Removal, replacement or making of hydroxyl groups increases the molecules lipophilicity. This facilitates percutaneous absorption as well as glucocorticoid receptor binding activity.

By covering the skin with an occlusive dressing such as a plastic wrap, this effect can be heightened as much as 100-fold. The solubility of glucocorticoid in the vehicle also affects penetration into epidermis.

The condition of skin also affects bioavailability. Penetration of the applied drug is inversely proportional to the thickness of stratum

corneum. It increases if the drug is applied over an inflamed or diseased skin. Penetration also depends on the humidity and temperature of the environment. The stratum corneum may act as a reservoir for topical corticosteroids and this is dependent on topical corticosteroids concentration and formulation.

CLASSIFICATION

There are a number of topical corticosteroids available for use. There are two important systems of classification; one of which classifies topical corticosteroids into seven classes-VII has the mildest ones (Table 1). The other classification is the one adopted by the British National Formulary and employs a four-point scale of mild, moderate, potent and very potent, to classify these agents (Table 2).

TABLE. 1. CLASSIFICATION OF TOPICAL CORTICOSTEROIDS

Class	Drugs
Class I (Super-Potent)	Clobetasol propionate, Halobetasol propionate, Betamethasone dipropionate 0.05% ointment (optimized vehicle)
Class II (Potent)	Mometasone furoate ointment Halcinonide Fluocinonide
Class III (Potent)	Fluticasone propionate, Betamethasone dipropionate Cream (0.05%)
Class IV (Mild – strength)	Mometasone furoate cream (0.1%) Triamcinolone acetonide cream (0.1%) Betamethasone valerate foam (0.12%)
Class V (Mild – strength)	Flurandranolide 0.05%) Betamethasone dipropionate lotion (0.05%) Hydrocortisone butyrate (0.1%)
Class VI (Mild)	Desonide cream (0.05%) Fluocinolone acetonide 0.01% cream
Class VII (Mild)	Topicals with hydrocortisone, dexamethasone

Table 2. Classification used by British National Formulary

Potency	Drugs
Very Potent	Clobetasol propionate (0.05%), Fluocinolone acetonide (0.2%) Halcinonide (0.1%)
Potent	Betamethasone valerate 0.1% and 0.25% Mometasone furoate % Desonide %
Moderately potent	Beclomethasone dipropionate (%) Clobetasone butyrate 0.05%
Mild	Dexamethasone 0.1-0.2% Methylprednisolone acetate 0.25%

Both these classifications are good. But the author prefers the four point classification while choosing the corticosteroid preparation because of the ease of clinical application and predictability of effects as well as side effects.

MECHANISM OF ACTION

Glucocorticoids diffuse through stratum corneum barrier and through cell membranes to reach the cytoplasm of keratinocytes and other cells present in epidermis and dermis. Diffusion through stratum corneum is the rate limiting step in delivery of the drug. Once it reaches the cytoplasm, glucocorticoids binds to a specific receptor glucocorticoid receptor. The binding of the receptor to its ligand results in activation of

the receptor, which dissociates from other components of the tetrameric complex. The ligand bound receptor then enters the nuclear compartment and interacts with specific response elements on the genome, glucocorticoid response elements. This modulates transcription of numerous genes. In contrast to the positive gene regulation, glucocorticoids can supress gene activation by several mechanisms including interfering with function of transacting factors.

The above interactions lead to changes in the expression of a wide range of genes, resulting in diverse cellular effects, which includes suppression of production of inflammatory cytokines, inhibition of T-cell activation, changes in the function of endothelial cells, granulocytes, mast cells and fiborblasts and inhibition of proliferation.

The transcription activity of the steroid receptor seems likely to be regulated by an alternative isoform of the receptor known as glucocorticoid receptor β and it is an endogenous inhibitor of glucocorticoid action.

Lipocortin, interleukin (IL-1) and lymphokines such as IL-2 are some of the proteins induced by the steroid. Lipocortin is capable of inhibiting the A2 phospholipase and reducing liberation of arachidonic acid which in turn reduces inflammatory response.

IL-1 has a role in inflammation. The in-vitro exposure of human keratinocytes to hydrocortisone decreases the liberation of constitutive IL-1 as well as IL-1 induced keratinocytes to hydrocortisone decreases the liberation of constitutive IL-1 as well IL-1 induced by ultraviolet radiation. This would also explain the anti-inflammatory and anti-proliferation effect of the drug.

The glucocorticoids have an immune suppressive action for B-and T-lymphocytes as well as over monocytes/macrophages. They also cause depletion of the number of Langerhans' cells.

SIDE EFFECTS

The side effects of topical corticosteroids are inevitable;

Local side effects

- Skin atrophy.
- Contact allergy.
- Steroid roascea and periora dermatitis.
- Rise in intraocular pressure.
- Depigmentation/Hypopigmentation.

Systemic side effects

- Hypothalamopituitary adrenal axis.

PAEDIATRIC USE

Topical corticosteroids are used with caution in children. The general principle is to use as mild a steroid as possible, for as short a period as possible, over as small area as possible.

USE IN PREGNANCY

Appropriate human studies using topical corticosteroids in pregnancy have never been undertaken. However, numerous pregnant patients are using glucocorticosteroids throughtout pregnancy without any problem to the infant.

FORMULATIONS

There are a number of topical corticosteroids preparations available in the market.

The topical corticosteroids for dermatological use are available as ointments, creams, lotions, gels and foam. Ointments enhance the percutaneous absorption of topical corticosteroids by increasing hydration and temperature of skin. Creams use emulsions of water-in-oil or oil-in-water with the active agent dispersed between oil and water phase. They

do not retard loss of heat or feel greasy. Lotions are a mixture of powder and liquid in an aqueous or alcoholic suspension, and gels are transparent semisolid emulsions that liquefy on contact with warm skin, drying as a greaseless, non-occlusive film.

COMBINATIONS

Several antimicrobials are commercially formulated in combination with topical corticosteroids. The usual drugs used are antibiotics and antifungals.

DILUTING READY MADE PREPARATIONS

The practice of diluting ready made preparations with another agent is not a welcome practice. With dilution, the bioavailability of a topical corticosteroids may be altered. Dilution may also introduce contamination.

TOPICAL STEROIDS IN CLINICAL PRACTICE

To have an appropriate response, the drug must be used judiciously as well as for sufficiently longer period. It is often observed that there is a premature withdrawal of topical corticosteroids. This will result in relapse of the disease.

PRACTICAL POINTS

- Use topical corticosteroids judiciously.

- Use steroid for sufficiently longer time so that there is remission of the condition.
- Wean –off steroid if used for more than one month.
- Give steroid holiday.
- For thick lesions – steroid + salicylic acid.
- For flexors – moderately potent steroid; avoid highly potent steroid.
- For hairy region – solution/foam.
- Avoid topical corticosteroids over oozing lesion.
- For subacute dermatitis – creams.
- For Chronic lichenified lesion – ointment.
- For Children – as mild a steroid as possible, for as short a period as possible, over as small an area as possible.

CHAPTER VI

LITERATURE REVIEW

1) A.J. Baillie and A.T. Florence et.al., formulated Niosomes by hydrating a mixture of single (or) double alkyl chain non ionic surfactant with cholesterol. They found that these vesicles can retain water soluble solutes such as carboxyfluorescein and release the entrapped solute slowly. The vesicles were prepared by Ether injection, Hand shaking and Sonication methods. The vesicles size were characterized by Proton correlation spectroscopy[33].

2) A.R. Mullaicharam and R.S.R. Murthy formulated and optimized Rifampicin Niosomes. They studied about the effect of change in process variable such as volume of solvent, hydration time, volume of hydrating medium and sonication time were studied. The prepared Niosomes were characterized for size, shape and lamellarity[34].

3) S.Bhaskaran and L. Panigrahi formulated and evaluated Niosomes using different non-ionic surfactants. Such as Span 20,40,60,80, Tween 20, 40, 60, 80 and Brij 35, The vesicle size were characterized by IR spectroscopy. They found that among all span 60 showed maximum release in 24h[35].

4) P.M. Sattur war et.al., formulated and evaluated ketoconazole Niosomes by Ether injection method. The prepared Niosomes were characterized for size, shape, entrapment efficiency and invitro drug release. In-vivo antifungal activity was also tested in rabbits[36].

5) H.E.J. Horland et.al., studied about the safety aspects of Niosomes. Two different toxicity models were used to assess the relationship between the physiochemical properties were described in terms of HLB values and CMC. Neither the HLB nor the CMC values appeared to have an effect on the safety of Niosomes as observed in both toxicity models[37].

6) Yongmei Hao et.al., studied about the best surfactant among span series in the aspect of encapsulation capacity. They used colchicines as a model drug. They found span 60 is the most ideal surfactant among all the four by surfactants in span series[38].

7) J.N.Khandare and G.Madhavi formulated and evaluated ketoprofen Niosomes. They used Non-ionic surfactants such as Tween 20, 40 and 80. Among the three they found Tween 40 showed more entrapment efficiency in-vivo anti-inflammatory activity was studied by Paw oedema method[39].

8) S.K shah et.al., prepared and evaluated miconazole nitrate Niosomes. They found Hand shaking method is a ideal one. And also Niosomal gel is better than plain gel. They concluded that Niosomal gel releases the drug slowly and increase retention time and this may restrict the entry of drug into systemic circulation[40].

9) Alok Namdeo et.al., formulated and evaluated the Niosomally encapsulated Indomethacin. Size of the Niosomes were characterized by light and scanning microscope. The Anti-inflammatory activity was studied by Paw-oedema method[41].

10) Agrawal sunil et.al., studied about the Niosomal Daunorubicin's anti-cancer activity in swiss mice bearing fibrosarcoma. Niosomes were prepared by Solvent dispersion method. The formulated Niosomes were characterized for size, entrapment efficiency and in-vivo drug release. Finally they proposed that encapsulation of daunorubicin in Niosomes is a potential tool for the effective delivery of drug[42].

11) D.Saravanan and H.Popli prepared and evaluated Metronidazole loaded Niosomes in rats. Niosomes were prepared by Hand Shaking method. Higher concentration of metronidazole were achieved in liver when the drug was entrapped in Niosomes and

the Niosomes could be considered as potential carriers for hepatic localization of Metronidazole[43].

12) M. carafa et.al., prepared and evaluated the properties of Niosomes. Niosomes were prepared by Polysorbate 20 and Cholesterol by using two different methods such as Direct Sonication and Thin film hydration method. They found higher entrapment efficiency in Thin film hydration method. Vesicles were characterized for vesicle dimension, entrapment efficiency and stability[44].

13) J.N. Khandare et.al., discussed about the disadvantages of liposomes, advantages of Niosomes and factor affecting drug entrapment and release characters[45].

14) S. Agarwal et.al., studied about the effect of cholesterol content and surfactant on vesicle properties of Niosomes. Niosomes were prepared using Primaguine phosphate as a model drug by mechanically shaking method without sonication. The Entrapment efficiency was increased with increasing cholesterol content. Mean size of Niosomes were also increased in increasing HLB value (span 85 → span 20)[46].

15) K. Ruckmani et.al., formulated Niosomes using Cytarabine Hydrochloride. The Niosomal vesicles were formulated using

Lipid hydration method. The sizes were obtained ranging from 600 to 1000 nm. The entrapment efficiency was high upto 80% with Tween 80, 20 and Span 60. The physical stability of Niosomes were good over a period of 4 weeks[47].

16) Ambikanandan Misra et.al., studied about the topically applied Nimesulide Niosomes were formulated using Lipid film hydration technique. Prepared Niosomes were optimized for high entrapment efficiency. Then the Niosomes were incorporated into gel and Anti – inflammatory activity was checked by Paw oedema method. Niosomally entrapped Nimesulide shows highest paw edema inhibition[48].

17) V.Ravichandran et.al., prepared and evaluated the in vitro release of Diclofenac sodium niosomes. Niosomes were prepared by Hand Shaking method and Ether injection method. The Niosomes were subjected for vesicle size determination and entrapment efficiency. Hand Shaking method showed high entrapment efficiency when compare to Ether injection method[49].

18) N.K.Jain and Manjusha malhotra discussed about various method of preparation of Niosomes, in vitro characterization such as vesicle diameter, entrapment efficiency, osmotic shrinkage, drug

release and route of administration including applications of Niosomes[50].

19) C.P. Jain et.al., studied about Niosomal drug delivery of Rifampicin to lymphatics. The Niosomes were prepared by Thin film hydration method. The vesicles were characterized for shape, size, entrapment efficiency and in vitro drug release. They proposed Rifampicin encapsulated in Niosomes could successfully used in the treatment of Tuberculosis[51].

20) J.N.Khandare et.al., prepared and evaluated Nimesulide niosomes for topical application. Nimesulide was enrtrapped in Niosomes prepared by various surfactant cholesterol ratio's by Ether injection method. Anti – inflammatory activity was evaluated by Paw oedema method. The niosomal gel show better activity over marketed formulation[52].

21) Toshimitsu Yoshika et.al., prepared and studied about the properties of vesicles of Span 20,40,60, 80 and 85. Niosomes were formulated using Mechanical shaking technique without sonication. Carboxfluorescein (CF) was used as model solute to investigate entrapment efficiency and release. Most efficient entrapment efficiency was shown with Span 60[53].

22) Maria Manconi et.al., prepared Tretinoin loaded Niosomes. The Niosomes were prepared from polyoxyethylene (4) lauryl ether, sorbitan ester and a commercial mixture of octy1/decy1 polyglucosides in the presence of cholesterol and phosphate. A study was done on the side, entrapment efficiency and in vitro drug release[54].

23) N.Udupa et.al., studied about the Anti-inflammatory activity of Niosome encapsulated Diclofenec sodium in rats. Niosomes prepared by using various Non-ionic surfactants such as Span 40,60,80,Tween 40,60,80 and Brij 35 with cholesterol. The Niosomes were subjected for size and invitro diffusion studies. The Anti-inflammatory activity was checked by Paw oedema method Intra-peritonelly injected Niosomes showed high paw oedema reduction than other routes[55].

24) N.K. Jain et.al., studied about the salient features of niosomes including in-vivo behaviour and in-vivo characterization such as vesicle diameter, entrapment efficiency and in-vitro drug release[56].

25) R. Agarwal et.al., prepared and evaluated Niosomally entrapped dithranol. Niosomes were prepared by using span 60 and

cholesterol. In vitro permeation studies were done by using mouse abdominal skin[57].

26) Jia-you fang et.al., studied about the effect of Niosomes on skin permeation of enoxacin. The invitro permeation studies were done by franz diffusion cells. Niosomal enoxacin shows higher stability when compare to liposomes[58].

27) C.O. Rentel et.al., studied about Niosomes as a novel peroral vaccine delivery system. The test was carried out by using BALB/c mice. Ovalbumin was encapsulated in various niosome preparations. Two different formulations were studied. Only ecapsulation of ovalbumin into wasag 7 (70% stearate sucrose ester, 30% palmitate sucrose ester (40% mono, 60%, di/trimester) Niosomes show significant increase in antibody titres.

28) A.T. Florence et.al., studied about the extrusion of Niosomes from capillaries. This was an approach for a pulsed drug delivery device. Niosomes were extruded from glass capillaries using air pressures of 0.5-5 PSI. The extrusion was affected by size, shape and membrane composition of Niosomes[59].

29) Anna M. Fadda et.al., studied about Niosomes as drug carriers for Tretinoin. Tretinoin niosomes were prepared by using Span 40,

60, Brij 30 and Triton CG110. Photostability of tretinoin was checked and tretinoin niosomes prepared by Brij 30 and triton CG110 showed retardation in the drug photodegradation when compare to tretinoin niosomes prepared by using span[60].

30) Behrooz Nasseri et.al., studied about the elastic properties of Niosomal membrane when there is change in temperature and elastic properties. Span 60 and cholesterol were used for Niosomal preparation. The proposed that surface elasticity was increased in increasing cholesterol content to some extent (around 40 mol% cholesterol) and further increase decreses the surface elasticity[61].

31) Ahmed S Guinedi et.al., prepared and evaluated multilamellar Niosomes as ophthalmic carriers of acetazolamide. Niosomes were studied to see the improvement in law corneal penetration and bioavailability character's Niosomes were prepared by Span 40, 60 with cholesterol. The prepared Niosomes were subjected to size analysis, entrapment efficiency and invitro drug release. The prepared Niosomes were subjected to size analysis, entrapment efficiency and invitro drug release. Span 60 Niosomes shows higher entrapment efficiency and shown prolonged decrease in intra ocular pressure[62].

32) Christine Dufes et.al., prepared glucose – targeted Niosomes to deliver Vasoactive intestinal peptide (VIP) to the brain. VIP/125I VIP – loaded glucose bearing Niosomes were intravenously injected to mice. Brain uptake was determined by r – counting. HPLC analysis confirmed the presence of VIP in brain after administration of VIP loaded Niosomes. In conclusion glucose bearing vesicles might be therefore a novel tool to deliver drugs across the blood brain barrier[63].

33) Abbar Pardakhty et.al., prepared and evaluated in-vitro study of Brij Niosomes for delivery of insulin. Niosomes were prepared by Film hydration method. Entrapment of insulin in bilayers protect it against proteolytic activity of α -chymotrypsin, trypsin and pepsin in vitro. Maximum protection was seen in Brij 92 Niosomes. The results indicate that Niosomes could be developed as sustained release oral dosage forms for delivery of peptides and proteins such as Insulin[64].

34) S.P. Vyas et.al formulated Niosomes for non-invasive topical genetic immunization against hepatitis-B. Here Niosomes for topical DNA delivery was studied. DNA encoded Hepatitis B surface antigen was encapsulated in niosomes composed of span 85 and cholesterol were prepared by Reverse phase evaporation

method. Niosomes were characterized for size, shape and entrapment efficiency. The study signify the potential of Niosomes as DNA vaccine carriers for effective topical immunization[65].

35) Elisabetta Gianasi et.al., formulated Niosomes and evaluated the biologically characterization of a Doxorubicin – polymer conjugate (pki). Niosomes were prepared using span 60, cholesterol and solulan. Niosomes were photographed by using optical and transmission electron microscopy. Final result showed Niosomes are potential for use in targeted cancer chemotherapy[66].

CHAPTER - VII

AIM AND PLAN OF THE WORK

"If you've ever suffered from the irritating itch of a skin disorder take heart… you're not alone." The above sentence is popular in the world now because every year millions of people suffer from some kind of skin disorder[67].

Glucocorticosteroids are used topically for a large variety of dermatological conditions. They benefit by virtue of their anti inflammatory, immune suppressive, vasoconstrictor and antiproliferative actions[31,68].

However, even though glucocorticoids can be used to treat vast skin disorders it also have some side effects. Moreover the premature withdrawal of corticosteroids will result in relapse of the disease. So less frequent application of a superpotent corticosteroid in a suitable carrier may have prolonged action with less or minimal side effects[69].

To achieve the above aim, niosome is one of the right choice. Since they can entrap both hydrophilic and lipophillic drugs, it can be employed as a suitable drug carrier for steroidal drugs.

According to structure activity relationship of steroidal drugs addition of fluorine atom at position 6 and/or 9 enhance the potency of steroid. 17th hydroxy group is also important to glucocorticoid activity. So glucocorticosteroid Clobetasol propionate is choosen.

Then various Clobetasol propionate niosomes were prepared by altering the ratios between various non ionic surfactants (span series) and cholesterol by three methods such as Thin film hydration method, Ether injection method and Hand shaking method.

The prepared niosomes were subjected to drug content analysis, Entrapment efficiency and Invitro drug release studies. The best niosomal preparations were subjected to size analysis by scanning electron microscopy and it is formulated as gel. Finally the niosomal gel and marketed gel were subjected to invitro drug release studies and invivo animal studies.

PLAN OF WORK

1. Calibration curve for the drug (Clobetasol propionate)
2. Preparations of Niosomal formulations using various ratios of non ionic surfactant and cholesterol by three methods.
    a) Thin film hydration method
    b) Either injection method and
    c) Hand shaking method
3. Determination of entrapment efficiency
4. Observation of vesicle size by scanning electron microscopy
5. In – vitro diffusion studies of clobetasol propionate niosomes
6. Formulation of the best niosomal formulation into gel form for topical drug delivery.
7. Invitro diffusion studies of niosomal gel preparation, plain gel and marketed gel preparation.
8. Stability studies of the best niosomes preparation from each method.
9. In-vivo animal studies to check the Anti-inflammatony effect in niosomal gel and marketed gel.

CHAPTER - VIII

MATERIALS AND EQUIPMENTS

MATERIALS USED

1.	Drug – Clobetasol Propionate	⇒	Apex laboratories
2.	Cholesterol	⇒	S.D fine – chem. Ltd
3.	Sorbitan mono palmitate (Span 40)	⇒	S.D. fine – chem. ltd
4.	Sorbitan mono stearate (span 60)	⇒	S.D. fine – chem. ltd
5.	Sorbitan mono oleate (span 80)	⇒	Loba chemie pvt ltd
6.	Chloroform	⇒	Vinbiotech systems (India)
7.	Methanol	⇒	Abstron Chemicals (India)
8.	N-Propanol	⇒	Lobachemie. Com
9.	Carbopol 934	⇒	Dr. Milton laboratories (Chennai)
10.	Diethyl either	⇒	TKM Pharma
11.	Triethanolamine	⇒	Robert Johnson
12.	Glycerol	⇒	Omega laboratory chemicals
13.	Propylene glycol	⇒	Robert Johnson

EQUIPMENTS USED

1.	Rotary flash evaporator	⇒	Super fit rotary flash evaporator modrobs
2.	Ultra sonicator	⇒	Vibronic's ultrasonic processor p2
3.	Electronic balance	⇒	A & D company, Japan
4.	Magnetic stirrer	⇒	MC Daldal & Co
5.	UV – Visible spectrophotometer	⇒	UV Pharma spec 1700 shimadzu
6.	pH meter	⇒	Dalal
7.	Scanning electron microscopy	⇒	JEOL JSM – 6360
8.	Refrigerator	⇒	Kelvinator
9.	Syringe and Needle	⇒	Dispovan

CHAPTER - IX

DRUG PROFILE

CLOBETASOL PROPIONATE[70,71,72,73,74,75,76]

GENERAL DESCRIPTION

CHEMICAL NAME

21 – chloro – 9 –Fluoro – 11 β – hydroxyl – 16 β methy1 3, 20 – dioxopregna – 1, 4 – dien-17 – y1 propanoate.

STRUCTURE

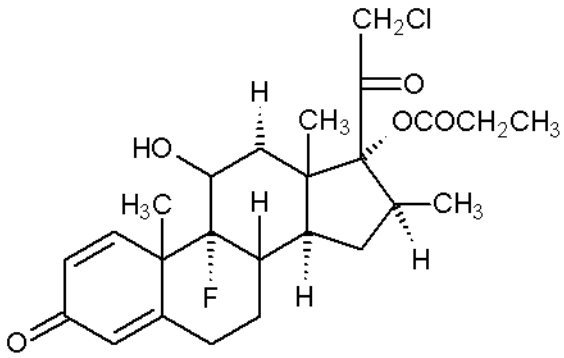


Figure – 7

MOLECULAR FORMULA

$C_{25}H_{32}CIFO_5$

MOLECULAR WEIGHT

467

CHIRALITY

The molecule has eight chiral centres

CATEGORY

Topical superpotent corticosteroid

PHARMACOLOGICAL ACTIONS

Anti – inflammatory, Anti – pruritic and Vaso constrictive properties.

USES

Alopecia areata;

Dermatitis, a topic, moderate to severe;

Dermatitis, exfoliative, generalized;

Dermatitis, nummular, moderate to severe;

Dermatoses, inflammatory, other, moderate to severe;

Dermatitis, other forms of, moderate to severe;

Granuloma annulare;

Keloids, reduction of association itching;

Lichen planus;

Lichen striatus;

Lupus erythenmatosus, discoid and subacute cutaneous;

Myxedema, pretibial;

Necrobiosis lipoidica diabeticorm;

Pemphigoid;

Pemphigus;

Pityriasis rosea;

Psoriasis;

Sarcoidosis; or

Sunburn

DOSAGE FORMS

Ointment, Gel, Foam, Cream, Shampoo, Solution

DOSE

Usual Adult Dose

Topically, to the skin as a 0.05% two (or) three times a day.

Usual Pediatric Dose

Children upto 12 years of age – use is not recommended

DESCRIPTION

White or almost white crystalline powder

SOLUBILITY

Practically insoluble in water, Freely soluble in acetone, sparingly soluble in ethanol (96%)

MELTING RANGE

Approximately 196^0C

SPECIFIC ROTATION

Between $+98^0$ and $+ 104^0$

LOSS ON DRYING

Not more than 2.0%

RESIDUE ON IGNITION

Not more than 0.1%

STORAGE AND PACKAGING

Store below 40^0C (104^0F) preferably between 15 and 30^0C (59 and 86^0F) in a well closed container

CLINICAL PHARMACOLOGY

Mechanism of Action

Clobetasol propionate acts by the induction of phospholipase A_2 inhibitory proteins collectively called lipocortins. Thes lipocortins control the potent mediators of inflammation such as prostaglandins and leukotrienes by inhibiting the release of their common precursor arachidonic acid.

PHARAMACOKINETICS

Absorption

Clobetasol propionate can be absorbed from normal skin. While inflammation and/or other disease processes in the skin may enhance percutaneous absorption.

Distribution

Following topical absorption only a small amount of drug appears to reach the dermis and subsequently to reach systemic circulation.

Metabolism

Presence of a substituted group in 17^{th} position and fluorinated group retards its local metabolism in skin.

Excretion

Clobetasol and its metabolites are excreted in bile and urine.

ADVERSE EFFECTS

Systemic Effects

Systemic effects may produce reversible hypothalamic – pituitary – adrenal axis, cushing syndrome, hyperglycemia and glucosuria.

Local Effects

Atropy of the epidermis, cracking cum tightening of skin, epidermal thinning, telangiectasia, purpura and atrophic striae may be seen.

PRECAUTION

Patient receiving large doses should be evaluated periodically for evidence of HPA axis suppression. The following tests are done to access the HPA axis suppression.

i) ACTH stimulation test

ii) A.M plasma cortisol test

iii) Urinary free cortisol test

CONTRA – INDICATION

Clobetasol propionate is contraindicated to patients having hypersensitivity to Clobetasol propionate or any component of the preparation.

CHAPTER – X

EXCIPIENT PROFILE

CHOLESTEROL[77]

SYNONYMS

Cholesterin, Cholesterolum

CHEMICAL NAME

Cholest -5- en-3β -ol.

EMPIRICAL FORMULA

$C_{27}H_{46}O$

MOLECULAR WEIGHT

386.67

FUNCTIONAL CATEGORY

Emolient.

Emulsifying agent.

DESCRIPTION

- Cholesterol occurs as white or faintly yellow, almost odurless, pearly leaflets, needles, powder or granules.
- On prolonged exposure to light and air, it acquires a yellow to tan color.

PROPERTIES

Boiling Point	360°C
Density	1.052 g/cm^3 for anhydrous form
Melting Point	147^{0}C - 150°C
Solubility	Soluble in acetone and vegetable oils. Practically insoluble in water. In chloroform, 1 in 4.5 at 20°C, In methanol, 1 in 294 at 0°C.

STABILITY AND STORAGE CONDITIONS

It is stable and should be stored in a well-closed container, protected from light.

SAFETY

It is generally regarded as an essentially non-toxic and non-irritant material at the levels employed as an excipient.

HANDLING PRECAUTIONS

Rubber or plastic gloves, eye protection and a respirator are recommended.

SORBITAN MONOPALMITATE[77]

SYNONYMS

Ablunol S-40, Armotan MP, Liposorb P, Span 40, Arlacel 40, Montane 40, Sorbitan Palmitate.

CHEMICAL NAME

Sorbitan monohexa decanoate.

EMPIRICAL FORMULA

C_{22} H_{42} O_6

MOLECULAR WEIGHT

403

DESCRIPTION

It occurs as cream solid with a distinctive odour and taste.

METHOD OF MANUFACTURE

Sorbitol is dehydrated to form a hexitan(1,4 Sorbitan) which is then esterified with the desired fatty acid.

PROPERTIES

Acid value	3 to 7
Hydroxyl value	270 to 303
Iodine valure	≤ 1
Density (g/cm^3)	1.0
HLB Value	6.7
Melting point	43^{0}C - 48^{o}C
Solubility	Soluble in oils and in most organic solvents. Insouble but dispersible in water.

FUNCTIONAL CATEGORY:

- Emulsifying agent.
- Non ionic Surfactant.
- Solubilizing agent.
- Wetting agent.

STABILITY

- Gradual soap formation occurs with strong acids or bases.
- Stable in weak acids or bases.

STORAGE

It should be stored in a well-closed container in a cool, dry place.

SAFETY

It is generally regarded as non-toxic and non-irritant material.

HANDLING PRECAUTIONS

Eye protection and Gloves are recommended.

SORBITAN MONOSTEARATE[77]

SYNONYMS

Ablunol S-60, Alkamuls SMS, Sorgen 50, Tego SMS, Span 60, Arlacel 60, Durtan 60, Montane 60, Sorbitan Stearate.

CHEMICAL NAME

Sorbitan mono – Octadecanoate.

EMPIRICAL FORMULA

$C_{24}\ H_{46}\ O_6$

MOLECULAR WEIGHT

431

DESCRIPTION

It occurs as a cream solid with a distinctive odour and taste.

METHOD OF MANUFACTURE

Sorbitol is dehydrated to form a hexitan(1,4 Sorbitan) which is then esterified with the desired fatty acid.

PROPERTIES

Acid value	5 to 10
Hydroxyl value	235 to 260
Iodine value	≤ 1
HLB Value	4.7
Melting Point	$53^0C – 57^oC$
Solubility	Soluble in oils and in most organic solvents. Insoluble but dispersible In water.

FUNCTIONAL CATEGORY

- Emulsifying agent.
- Nonionic Surfactant.
- Solubilizing agent.
- Wetting agent.

STABILITY

- Gradual Soap formation occurs with strong acids or bases.
- Stable in weak acids or bases.

STORAGE

It should be stored in a well-closed container in a cool, dry place.

SAFETY

- It is generally regarded as non toxic and non irritant material.

- Very mildly toxic by ingestion.

HANDLING PRECAUTIONS

Eye protection and Gloves are recommended.

SOBITAN MONO OLEATE[77]

SYNONYMS

Ablunol S-80, Armotan MO, Capmul O, Crill 4, Lamesorb SMO, Span 80, Arlacel 80, Montane 80, Sorgen 40, Sorbitan Oleate.

CHEMICAL NAME

(Z) - Sorbitan mono -9- Octa decenoate.

EMPIRICAL FORMULA

C_{24} H_{44} O_6

MOLECULAR WEIGHT

429

DESCRIPTION

It occurs as yellow viscous liquid with a distinctive odour and taste.

METHOD OF MANUFACTURE

Sorbitol is dehydrated to form a hexitan(1,4 Sorbitan) which is then esterified with the desired fatty acid.

PROPERTIES

Acid value	≤ 8
Hydroxyl value	193 to 209
Pour point	12
Density (g/cm^3)	1.01
HLB Value	4.3
Solubility	Soluble in oil and in most organic solvents. Insoluble but dispersible in water.

Soluble in oil and in most organic solvents. Insoluble but dispersible in water.

FUNCTIONAL CATEGORY:

- Emulsifying agent.
- Nonionic surfactant.
- Solubilizing agent.
- Wetting agent.

STABILITY:

- Gradual soap formation occurs with strong acids or bases
- Stable in weak acids or bases.

STORAGE:

It should be stored in a well-closed container in a cool, dry place.

SAFETY:

It is generally regarded as non-toxic and non-irritant material.

HANDLING PRECAUTIONS:

Eye protection and Gloves are recommended.

CARBOMER[77]

SYNONYMS

Acritamer, Carbopol, acrylic acid polymer, carboxy polymethylene, polyacrylic acid, carboxy vinyl polymer.

AVAILABLE GRADES

Carbomer 910.

Carbomer 934.

Carbomer 940.

Carbomer 971 P.

Carbomer 974 P.

MOLECULAR WEIGHT

7,00,000 to 4 billion

FUNCTIONAL CATEGORY

Bioadhesive.

Emulsifying agent.

Release-modifying agent.

Suspending agent.

Tablet binder.

Viscosity- increasing agent.

DESCRIPTION

Carbomers are White-colored, ‘fluffy’ acidic, hygroscopic powders with a slight characteristic odor.

PROPERTIES

Density	1.76 -2.08 g/cm^3
Melting Point	Decomposition occurs within 30 min at 260^{o}C
Moisture content	Normal water content is upto 2% w/w, hygroscopic. Typical equilibrium moisture content at 25^{o}C.
Solubility	Souble in water, after neutralization in ethanol (95%) and glycerin.
Specific gravity	1.41
Viscosity	Carbomers disperse in water to form acidic colloidal dispersions of low viscosity that when neutralized produce highly viscous gels.

STABILITY

Carbomers are stable, hygroscopic materials that may be heated at temperatures below 104ºC for upto 2 hours without affecting their thickening efficiency.

STORAGE

Carbomer powder should be stored in air tight, corrosion-resistant container in a cool, dry place. The use of glass, plastic or resin-lined container is recommended.

SAFETY

Carbomers are used extensively in non-parenteral products, particularly topical liquid and semi-solid preparations. It is generally regarded as essentially non- toxic and non- irritant materials.

HANDLING PRECAUTIONS

Carbomer dust is irritating to the eyes, mucous membranes and respiratory tract. Gloves, eye protection and a dust respirator are recommended during handling.

CHAPTER – XI

EXPERIMENTAL DETAILS

PREPARATON OF CALIBRATION CURVE

50 mg of drug was taken in a 50 ml standard flask and dissolved in a small amount of methanol. Finally the solution was made upto 50 ml with the same methanol.

Then 10 ml of the above solution was pipetted out into an another 100 ml standard flask and made up to 100ml by methanol.

Then from above solution 1,2,3,4,5….10 ml solutions were pipetted out into 10 different 100ml standard flasks and made up to 100 ml by distilled water.

Aliquant portions of solution was scanned in spectrophotometer to find λ max. Then the absorbance of various dilutions were measured at λ max (242 nm) in the UV-visible spectrophotometer, using distilled water as blank and standard curve was plotted by taking concentration in X-axis and absorbance in Y-axis.

PREPARATION OF CLOBETASOL PROPIONATE NIOSOMAL FORMULATIONS

Different ratios of surfactant and cholesterol were prepared as shown in Table no – IV A,B,C with same concentration of the drug.

Niosomes of Clobetasol propionate was prepared by three methods. They are as follows.

a. Thin film hydration method
b. Ether injection method and
c. Hand shaking method

A. Thin Film Hydration Method[79,80]

- Surfactant, cholesterol and drug were weighed separately and taken in to two different beakers.
- 15 ml of Chloroform: Methanol (2:1) mixture was added to the surfactant : cholesterol mixture and stirred until it gets completely dissolved.
- Then the above solution was added to drug and stirred until the drug gets completely dissolved.
- Now the above organic layer was introduced into the round bottom flask of Rotary flash evaporator by vacuum.

- The Round Bottom Flask was allowed to rotate at 180 rpm at 60^0C in a water bath until the organic layer was completely evaporated.
- After evaporation, the surfactant, cholesterol and drug formed a thin film on the inner sides of Round Bottom Flask.
- Then water was incorporated into round bottom flask by vacuum and allowed to vortexed for 1 hour.
- The thin film layer was peeled out and swelled in water to form Niosomes.
- The above white dispersion (Niosomes) was cooled in an ice bath and then sonicated using probe type ultrasonicator for 3 min at 150v.
- The resulted vesicles of niosomes were stored at 4^0C in a refrigerator.

Plain niosomes were also prepared without drug using the above procedure.

B. Ether Injection Method[81]

- Here the surfactant, cholesterol and drug were weighed separately and dissolved by using 10ml of Diethyl ether: Methanol (1:1) mixture.
- The above organic layer was taken in an 50 ml syringe having 14 guaze needle fitted in it.

- Then in an separate beaker adequate amount of distilled water was taken and it was placed over a magnetic stirrer and kept in a temperature between 55^0C and 65^0C.
- Aqueous layer was rotated by using a magnetic bead.
- The above organic layer was injected into swrilling aqueous phase at a rate of 0.25 ml / min.
- Vapourization of ether and methanol resulted in the formation of niosomes.
- Finally the niosomes were stored at 4^0C in a refrigerator.

Plain niosomes were also prepared without drug using the above procedure.

C. Hand Shaking Method[82]

- Here the surfactant, cholesterol and drug were weighed separately.
- 10ml of Chloroform: Methanol (1:1) mixture was added to surfactant, cholesterol mixture and stirred until it dissolved and the above solution was added to the drug and stirred until completely dissolved.
- The above organic layer was transferred to the round bottom flask by vacuum.

- The round bottom flask was vortexed at 60^0C until all the organic liquid was completely evaporated.
- After that adequate amount of the aqueous phase (Distilled water) was added and shaken by hands in an water bath at 60^0C until a white dispersion was formed (Niosomes).

Plain niosomes were also prepared without drug using the above procedure.

DRUG CONTENT ANALYSIS[79,83,84]

Drug content of the niosomal preparations were determined by lysis method. 50% n-propanol was used for lysing the niosomes. 1ml of the niosomal formulation was taken in 100ml standard flask. Then adequate amount of 50% n-propanol was added and shaken well until all the vesicles were completely lysed. The volume was made upto 100ml with distilled water. 10ml of the above solution was further diluted to 100ml with the same distilled water. Now the absorbance was measured at 242 nm by UV-spectrophotometer shimadzu using plain niosomes as blank.

ESTIMATION OF ENTRAPMENT EFFICIENCY[82,83]

Entrapment efficiency was found out by dialysis method. The Cellophane membrane was used as a semipermeable membrane. Here the cellophane membrane was soaked in Glycerol: water (1:3) mixture for 15 min. It was tied in an open ended tube and 2ml of Niosomal solution was transferred into it. The was is placed into a 250ml beaker containing 100 ml Distilled water and it was stirred by magnetic stirrer. The samples were taken every 15 min for 6 hours. The absorbance was measured at 242 nm by UV-spectrophotometer shimadzu using distilled water as blank and the entrapment efficiency was calculated by the following formula.

Entrapment efficiency = % Drug content - % of maximum drug release of unentrapped drug.

SIZE ANALYSIS OF NIOSOMES[82]

The niosomes were subjected to microscopic examination for characterization size and shape of the vesicles. The vesicles size of the prepared niosomes were analysed by Scanning Electron Microscopy (SEM).

IN-VITRO RELEASE STUDIES[85,80]

Drug Release studies of Clobetasol propionate was done in distilled water. The pH of normal health human is between 4.5 and 6. However with increase in age the skin pH becomes more and more neutral and thus more susceptible to bacterial growth and also the pH value rises beyond 6, when a person actually suffers from a skin problem (or) skin disease. So the Drug Release studies of Clobetasol propionate Niosomes were done in distilled water[78].

In vitro release studies were carried out by dialysis method. Cellophane membrane was soaked in Glycerin : water (1:3) mixture for 15 min. It was tied in an open ended tube and to this 1.5 ml of niosomal solution was added and then it was placed into a receptor compartment i.e; a 250 ml beaker containing 100 ml of distilled water and it was stirred by magnetic stirrer. The samples were taken periodically for 8 h.

The absorbance was measured at 242 nm by UV-spectrophotometer shimadzu (UV-1700 Pharma spec, Japan) using distilled water as blank to find the amount of drug release from the niosomal preparations. The percentage drug released was plotted against time to find the drug release behaviour of all niosomal formulations.

STABILITY STUDIES[86]

The best niosomal preparations were stored in two different temperatures such as $4^0C \pm 2^0C$ and $30^0C \pm 2^0C$ and the drug content was estimated every week for a period of 10 weeks and the results were tabulated.

FORMULATION OF VARIOUS GEL'S

From the in-vitro release studies, the best niosomal formulation was selected and incorporated into suitable gel base. The prepared Gel containing plain drug and drug incorporated in niosomes.

The gel base was prepared using the following formula.

Carbopol 934	-	2.0 %
Triethanolamine	-	1.65 ml
Purified water (freshly boiled and Cooled)	-	100 ml

Carbopol 934 was weighed and dispersed in 100 ml purified water at room temperature by constant stirring. The Triethanolamine was added and stirred until a viscous smooth gel was obtained.

A. Preparation of Plain Clobetasol Propionate Gel (G_1)[86]

The quantity of pure Clobetasol propionate (in methanol) equivalent to 0.1 % w/w (50 mg) was incorporated with 10 g of carbopol gel base by trituration and stirred by using a glass rod to get 0.05 % w/w of smooth homogenous Clobetasol propionate plain gel.

B. Preparation of Niosomal Clobetasol Propionate Gel ($G_{2)}$[86]

Formulation 1[Span 60: Cholesterol (1:0.5)] was selected for the preparation of gel. The selection was based on the results of entrapment efficiency. Since F1 has more entrapment efficiency when compared to all the other formulations, it was selected to prepare niosomal gel. Niosomal gel equivalent to 0.1% w/w concentration was incorporated to the gel base and stirred using glass rod to get 0.05% w/w of smooth homogenous Clobetasol propionate niosomal gel.

DRUG CONTENT STUDIES OF GELS[79,83]

A. Plain Clobetasol Propionate Gel:

The gel containing 1 mg of drug was weighed and transferred to an 100ml standard flask. To this a small quantity of distilled water was added and shaked well and made upto 100ml with distilled water. Then 10ml of the above solution was diluted to 100ml with the same distilled water.

The absorbance was measured at 242 nm by UV-spectrophotometer Shimadzu CUV-1700 pharma spec, Japan) using plain gel solution prepared by the above method as blank.

B. Clobetasol Propionate Niosomal Gel G_2:

The equivalent to 1mg of drug was weighed and transferred to 100 ml standard flask. The adequate amount 50% propanol was added and shaken well until the niosomes were lysed completely. Finally the solution was made upto 100 ml with distilled water. 10ml of the above solution was diluted to 100ml with the same distilled water.

The absorbance was measured at 242 nm by using UV-visible spectrophotometer shimadzu (UV-1700 pharma spec, Japan) using plain niosomal solution prepared by above method as blank.

INVITRO RELEASE STUDIES OF GELS (G_1, G_2, G_3) [80,85]

Invitro release studies was done in plain Clobetasol propionate gel, two Clobetasol propionate niosomal gels and marketed Clobetasol propionate gel.

Invitro release studies were carried out by dialysis method using cellophane membrane as a semi permeable membrane.

Cellophane membrane was soaked in Glycerin water (3:1) mixture for 15 min. Then it is tied in a open ended tube and 3 g of gel is transferred into it. The open ended tube was kept as donar compartment.

Then it was immersed into an 250ml beaker containing 100ml of distilled water and kept as recipient compartment.

The contents were uniformly rotated by a magnetic bead at 50rpm at 37^0c $\pm$ 2^0c. Then the samples were withdrawn at predetermined intervals. Finally the absorbance was measured at 242 nm by using UV-spectrophotometer shimadzu (UV-1700 pharma spec, Japan) using distilled water as blank. The percentage release was calculated and plotted against time. The percentage release of drug from all the gels was compared.

PHARMACODYNAMIC STUDIES OF GEL FORMULATIONS[79,85,89,90,91]

The Anti-inflammatory activity was carried out by paw oedema method.

MATERIALS AND METHODS

Animals : Albino rats of wistar strain (150-200g) of either sex were procured and kept in standard polypropylene cages and kept under controlled room temperature overnight. The rats were given a standard laboratory diet, water and libitium. Food was withdrawn 12 hours before and after the experimental hours.

Drugs

Plain Clobetasol propionate gel (marketed), Clobetasol propionate niosomal gel and carrageenanan.

Anti-Inflammatory Study

The animals were divided into three groups having four animals in each group. Group-I is kept as control without drug. For Group II and Group III Clobetasol propionate niosomal gel and plain Clobetasol propionate gel (marketed) were applied in the right hind paw of the rats respectively. After half an hour of the gel application for all the animals 0.1ml of 1% carrageenan in normal saline was injected in sub plantar on the same right hind paw of the rats. Then the paw oedema was measured by using plethysmometer at 0, 1, 2, 3, 4, 6 and 8 hours respectively.

Mean paw oedema was measured and percentage of inhibition was calculated.

CHAPTER – XII
RESULTS AND DISCUSSION

In the present study, eighteen formulations of Clobetasol propionate Niosomes were prepared by using non-ionic surfactants (Span 40,60 and 80) along with Cholesterol in different ratios of (S:C) (1:0.5 and 1:1) with the concentration of the drug being constant (5 mg) as shown in Table no – IV A,B,C.

CALIBRATION CRUVE

Calibration curve of Clobetasol propionate was plotted by measuring the absorbance of different concentrations of the drug in distilled water at 242 nm. The calibration curve was used to determine the drug content, entrapment efficiency, release studies and stability studies of niosomes. Clobetasol propionate obeys beer's law within the concentration range of 1 to 10 µg/ml and posses a λ max at U.V region of 242 nm. The results were present in Table - III and figure 8.

ENTRAPMENT EFFICIENCY

The entrapment efficiency of the prepared niosomal formulations was measured by dialysis method. The entrapment efficiency was determined by subtracting the amount of drug dialysed from the total amount of drug in the formulation and the values are given in

Table no – V and Figure 9A, 9B, 9C.

In all the formulations, the impact of Cholesterol, surfactant and method of preparations on entrapment efficiency was significant.

Effect of Cholesterol Content

The formulations with increased cholesterol ratio (F2, F4, F6, F8, F10, F12, F14, F16 and F18) showed decrease in entrapment efficiency compared with other formulations. The results are shown in Table - V and figure 9A, 9B, 9C. This revealed that the increase in cholesterol content decreases the entrapment efficiency which may be due to intercalation of cholesterol in the bilayers.[82]

Effect Of Surfactant

Among all the formulations F1 [span 60: cholesterol (1:0.5)] showed maximum entrapment efficiency compared with other formulations[35]. The entrapment efficiency increases in the order of **span 60 > span 40 > span 80**. The results are shown in Table V and figure 9A, 9B, 9C.

The results showed that entrapment efficiency increases with decrease in HLB value of non-ionic surfactant with an exception of span 80 because it has unsaturated alkyl chain compared to span 60.[80,53]

Further span 60 showed higher entrapment efficiency than the span 40 due to the higher phase transition temperature of Span 60 than that of Span 40.[83]

Effect Of Method

Among all the three methods Thin film Hydration method showed maximum entrapment efficiency compared with other formulations. The entrapment efficiency increases in order of **Thin film hydration method > Hand shaking method > Ether injection method**. The results are shown in Table no: V and figure 9A, 9B, 9C.

The amount of entrapment efficiency by Hand shaking method was more than ether injection method. This may be due to vortexing only which was carried out during hand shaking method, whereas in the case of ether injection process vortexing and injection take place simultaneously. Moreover the vesicles obtained by hand shaking method were larger when compared to vesicles obtained by ether injection method. So due to this entrapment efficiency was more in hand shaking method when compared with ether injection method[49].

The entrapment efficiency of the Thin film hydration method was more, when compared with hand shaking method. This may be due to uniform and mechanical vortexing in Thin film hydration method.

SIZE ANALYSIS OF NIOSOMES

The vesicle size of the prepared niosomes were observed and measured by Scanning Electron Microscopy. Most of the vehicles were found to be spericalin shape and in size ranges from 50 to 200 nm. However the size of niosomes in Hand shaking method was larger when compared with the Thin film hydration method and ether injection method.

INVITRO RELEASE STUDIES

The invitro release studies of all span niosomal formulations were carried out by diffusion method and the percentage of drug released from these formulations are shown in table no: VI A, B, C and Figure 10A, B, C

A. Effect of Entrapment Efficiency

a. Thin Film hydration method

Among all the six formulations from F1 to F6, F1 shows maximum drug release and the order of decreasing percentage drug release in 24^{th} hour are F1 (49.76%) > F5(42.44%) > F6 (36.09%) > F4 (32.69%) > F3 (28.41%) > F2 (22.30%) The results are shown in Table VI-A and Figure 10A.

b. Ether injection method

Among all the six formulations from F7 to F12, F10 shows maximum drug release and the order of decreasing percentage of drug release in 24th hour are F10 (42.44%) > F11 (39.70%) > F9 (37.06%) > F8 (35.17%) > F7 (30.93%) > F12 (27.43%). The results are shown in table VI-B and Figure 10B.

c. Hand shaking method

Among all the six formulations from F13 to F18, F13 shows maximum drug release and the order of decreasing percentage drug release in 24th hour are F13 (41.78%) > F15 (36.66%) > F17 (35.76%) > F18 (32.21%) > F14 (28.93%) > F16 (26.68%) The results are shown in table VI-C and 10C.

B. Effect of Surfactant And Cholesterol.

a. Thin film hydration method

Among all the six formulations from F1 to F6, F1 shows maximum drug release[35] and the order of decreasing percentage drug release in 24th hour are [S60: Chol (1:0.5) – 49.76%] > [S80 : chol (1:0.5) – 42.44%] > [S80:chol (1:1) - 36.09%] > [S40:chol (1:1) - 32.69%] > [S40:chol (1:0.5) – 28.41%] > [S60:chol (1:1) – 22.30%]. The results are shown in Table VI A and Figure 10A.

b. Ether injection method

Among all the six formulations from F7 to F12, F10 shows maximum drug release and the order of decreasing percentage drug release in 24^{th} hour are [S40:chol (1:1) – 42.44%] > [S80:chol (1:0.5) 39.70%] > [S40:chol (1:0.5) - 37.06%] > [S60:chol (1:1) – 35.17%] > [S60:chol (1:0.5) -30.93%] > [S80:chol (1:1) – 27.43%]. The results are shown in table VI-B and Figure 10B.

C. Hand Shaking Method

Among all the six formulations from F13 to F18, F13 shows maximum drug release and the order of decreasing percentage drug release in 24^{th} hour are [S60:chol (1:0.5) – 41.78%] > [S40:chol (1:0.5) – 36.66%] > [S80:chol (1:0.5) – 35.76%] > [S80:chol (1:1) – 32.21%] > [S60:chol (1:1) – 28.93%] > [S40:chol (1:1) – 26.68%]. The results are shown in table VI-C and figure 10C.

STABILITY OF NIOSOMES

The stability of the best formulation in each group of surfactant (High entrapment efficiency) F1, F7 and F13 were carried out by storing at $4^0C \pm 2^0C$ (refrigeration temperature) and $30^0C \pm 2^0C$ (room temperature) for 10 weeks.

The retention of drug in niosomal formulations was calculated immediately after the preparation and was taken as 100% retention. The percentage of drug retention in the niosomal preparation at various time intervals was determined. The results are shown in table no: VII-A, VII-B and figure 11a, 11b and 11c.

The results showed that the drug retention capacity was at $4^0C \pm 2^0C$ but increase in temperature and storage period decreases the drug retention capacity.

GEL FORMULATION

The selected best niosomal formulation (on the basis of highest entrapment efficiency among all the three methods) was incorporated into suitable gel base (carbopol) to obtain 0.05% of the drug and plain Clobetasol propionate gel was prepared by incorporating the drug into suitable gel base to obtain same 0.05% of the drug. Both the formulas were tabulated in Table VIII A and VIII B.

Drug Content

The drug content of the prepared gels was determined after lysing the vesicles. It was estimated spectrophotometricaly at 242 nm in UV-visible 1700-pharma spec, Japan. The results are shown in Table IX. The uniform distribution of drug in all the gel formulations was observed.

In-Vitro Release Studies

In vitro release studies were performed for the gels prepared with the carbopol base and compared with the marketed product (0.05% gel)

From the results shown in table – X marketed product (G3) showed 98.43% release in 300 min. The carbopol gel containing pure drug (G1) showed 99.12% release in 360 min as shown in Table X and figure 12. In case of carbopol gel containing niosomes showed 51.58% release in 24th hour. This shows the release of drug was controlled due to the entrapment of drug in vesicles. The results are shown in Table X and figure 12. The increase in order of release is **G3 > G1 > G2**.

PHARMACODYNAMIC STUDY OF GEL FORMULATIONS[90,89]

The pharmacodynamic study was carried out to find out the anti-inflammatory activity of niosomal gel preparation (G2) and to correlate it with marketed gel formulation. It was assessed by carrageenan induced hind paw oedema method in white albino rats.

After Studying the anti inflammatory activity, the percentage inhibition of oedema by clobetasol propionate niosomal gel and marketed gel were compared with their respective control groups, statistically by students ‘t’ test. The results were shown in Table XI-A, XI-B and Figure 13.

The percentage of reduction in paw oedema was gradually increasing in the case of niosomal gel upto 8^{th} hour and whereas in marketed gel the percentage of reduction in paw oedema was gradually increasing upto 4^{th} hour and later it declined on 6^{th} and 8^{th} hour.

The above results showed niosomal gel had a sustained as well as prolonged action when compared with marketed gel.

CHAPTER XIII
SUMMARY AND CONCLUSION

- Clobetasol propionate niosomes were prepared using surfactants span 40, 60 and 80 in two different ratios by three methods.
- The niosomal formulations were characterized for their vesicle size, entrapment efficiency and invitro release study.
- The entrapment efficiency was found to be highest for niosomal formulation (F1) when compared with other niosomal formualtions.
- The shape of Clobetasol propionate niosomes are spherical in nature and the size ranges between 50 and 200 nm.
- The invitro release study showed prolonged release profile for all the eighteen niosomal formulations and F1 showed more drug release at 24^{th} hour.
- On the basis of entrapment efficiency and invitro release study, the niosomal formulation (F1) equivalent to 0.05% w/w of clobetasol propionate was incorporated into carbopol gel base and evaluated with 0.05% w/w of plain clobetasol propionate gel and marketed gel having same 0.05% of clobetasol propionate. The gels were easy to prepare and found to be homogenous in composition.

- ❖ The invitro release studies of clobetasol propionate gel formulations revealed that the release rate of clobetasol propionate from niosomal gel was slower than that of plain clobetasol propionate gel and marketed gel.
- ❖ Pharmacodynamic activity of clobetasol propionate gel showed uniform and prolonged anti-inflammatory activity when compared to marketed gel preparations. Therefore the present study suggests the potentiality of noisomes to enhance the therapeutic effect and minimize the side effects of clobetasol propionate.

From the above study it is concluded that the niosomes can be considered as promising drug delivery vehicles which increase the duration of action of the drugs. Thus niosomes help in formulating dosage forms having more prolonged action. The niosomal delivery of clobetasol propionate in carbopol gel base acts as a suitable topical drug delivery system which may be given safely to all patients suffering from skin disorders (above 12yrs). The gel produces smoothness and cooling effect with more compatibility with the skin surface.

Further the pharmacokinetic and other clinical studies may be carried out in future.

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